REMARKS

Claims 12-32 are pending in the present application. Claims 10 and 11 have been cancelled, and new claims 21-32 have been added. Claims 12, 16 and 20 are amended to correct grammatical errors and an error in antecedent basis, and to remove unnecessary verbiage. Claim 12 has been additionally amended to recite a culturing step. Claims 13-15 and 17-18 have been amended to delete reference to cancelled claim 10, and/or to clarify that the concentration of dissolved oxygen recited is in the medium in which the cells are cultured [(b)]. The specification has been amended to insert the title of the paragraph containing the priority information ("CLAIM OF PRIORITY"), and to indicate that the parent application (09/478,163) has been abandoned. Support for the amendments can be found throughout the application as filed, including the claims, especially at p. 4, line 16 – p. 5, line 11; p. 6, line 3 - p. 7, line 8, and p. 8, lines 27-29, *inter alia*. No new matter has been added.

Objection to the Specification

The Examiner objected to the specification for lacking a specific reference to prior applications. Applicant has previously amended the specification to insert a paragraph regarding the prior applications, in the Preliminary Amendment submitted with the application on November 9, 2001 (which Amendment is referred to by the Examiner on page 2 of the present Office Action). The paragraph has now been updated to reflect the current status of the prior application. Applicant submits that the claim of priority is perfected in compliance with 35 U.S.C. 120 and 37 C.F.R. 1.78, and requests withdrawal of the objection to the specification and acknowledgement of the claim of priority.

Information Disclosure Statements

The Examiner noted that reference AL of the information disclosure statement submitted on January 28, 2002 was incompletely referenced. The patent is Japanese Patent No. 2977241, filed August 1, 1990; the application, number 02-202616, was published on April 2, 1991 as Publication No. 03-076595. A new form 1449 with additional information regarding that

reference is attached, as well as an English translation of the abstract and a summary of the document, and an English-language equivalent, Irish Patent No. 65040. Applicant requests that the Examiner initial the references on the enclosed form 1449, and return a copy of the initialed form to the Applicant.

In addition, Applicant notes that reference AA (U.S. Pat. No. 5,763,236) of the information disclosure statement filed on May 14, 2002 was not initialed by the Examiner. Applicant hereby requests that the Examiner initial reference AA on the previously-submitted form 1449, and return a copy of that initialed form to the Applicant.

Claim Objections

Claims 10 and 12 were objected to by the Examiner for lacking clarity. Claim 10 has been cancelled, and claim 12 has been amended in accordance with the Examiner's suggestions. Applicant therefore requests withdrawal of the objections thereto.

Rejections Under 112, Second Paragraph

The Examiner has rejected claim 12 (and claims 13-20 dependent thereon) for allegedly being indefinite. Specifically, the Examiner objects to the term "racemate" because it lacks antecedent basis. Claim 12 has been amended in accordance with the Examiner's suggestion to replace the term "racemate" with "racemic alcohol." Applicant submits that this amendment obviates the rejection under 35 U.S.C. § 112, second paragraph, and requests withdrawal thereof.

Rejections Under 112, First Paragraph

The Examiner has rejected claims 10-20 for failing to meet the written description and enablement requirements of 35 U.S.C. § 112, first paragraph. Applicant respectfully traverses, for the following reasons.

Written Description

The Examiner states that

> While the specification discloses the culturing of E. coli JM109 [pKK-CPAl], wherein pKK-CPAl comprises a gene from *C. parapsilosis* encoding a secondary alcohol dehydrogenase, at low dissolved oxygen concentrations (Examples 4 and 5), and the increase in specific alcohol dehydrogenase activity at low dissolved oxygen

concentrations (Table 1 and Table 2), the specification fails to provide any information regarding (1) the specificity of the alcohol dehydrogenase encoded in pKK-CPA1, (2) other alcohols which can be oxidized by the alcohol dehydrogenase encoded in pKK-CPA1, (3) other oxidoreductases from other organisms, the identity of those organic compounds which can be oxidized by the oxidoreductases, and their corresponding electron acceptors, (4) other oxidoreductases from *C. parapsilosis* which can be used to oxidize any organic compound or to produce optically active alcohols, (5) the electron acceptors of the oxidoreductases of (4), and (6) other oxidoreductases from other organisms which specifically oxidize either the (S) or (R) enantiomer in racemic alcohol mixtures, the identity of those alcohols which can be oxidized by these reductases, and their corresponding electron acceptors. The specification fails to describe other representative species of the genus of oxidoreductases, alcohol dehydrogenases, organic compounds, alcohols, electron acceptors, and *C. parapsilosis* oxidoreductases required to practice the claimed method. (Office Action, pp. 5-6)

Further, the Examiner states

The specification provides one oxidoreductase from *C. parapsilosis,* one organic compound, 1,3 butanediol, and 5 electron acceptors, which is insufficient to put one of ordinary skill in the art in possession of all attributes and features of the claimed method. Therefore, one skilled in the art cannot reasonably conclude that the applicant had possession of the claimed invention at the time the instant application was filed. (Office Action, page 6)

It is well accepted that a specification may satisfy the written description requirement of 35 U.S.C. §112 for a given genus claims without describing every species that the claim encompasses. The Federal Circuit in Enzo Biochem, Inc. v. Gen-Probe Inc., 296 F.3d 1316 (Fed. Cir. 2002) stated that

> the written description requirement can be met by "show[ing] that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics." 296 F.3d at 1324 (emphasis omitted, bracketed material in original).

Thus, the issue of whether or not the written description requirement is met is factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure. Possession of a genus can be evidenced by describing the distinguishing identifying characteristics common to the divergent species encompassed.

In this case, the invention is directed to methods for enhancing the efficiency of a specific kind of redox reaction that is catalyzed by an enzyme generically known as an "oxidoreductase." The genus of oxidoreductases is a recognized class of enzymes:

> **Oxidoreductase** (ox·i·do·re·duc·tase) [EC 1] any member of a class of enzymes that catalyze the reversible transfer of electrons from a substrate that becomes oxidized (hydrogen or electron donor) to a substrate that becomes reduced (hydrogen or electron acceptor). The class includes dehydrogenases, hydroxylases, oxidases, oxygenases, peroxidases, and reductases. (Dorland's Illustrated Medical Dictionary, W. B. Saunders, 2002)

Many oxidoreductases have been produced recombinantly and/or purified from natural sources. As a group, they are well characterized, and reactions associated therewith are well understood by those of skill in the art. Entire books had been written about oxidoreductases at the time this application was filed; see, e.g., Bergmeyer et al., Eds., Methods of Enzymatic Analysis, Enzymes 1: Oxidoreductases, Transferases (John Wiley & Sons Inc; 3rd edition (1983)), and the Enzyme Handbook 6: Class 1.2 - 1.4 Oxidoreductases, Schomburg et al., Eds., (Springer-Verlag 1993); Enzyme Handbook 7: Class 1.5 - 1.12 Oxidoreductases, Schomburg et al., Eds. (Springer-Verlag 1994), *inter alia.* Oxidoreductases catalyze the transfer of an electron from a substrate having an electron donor moiety, typically a carbon-OH bond, an aldehyde group, a carbon-carbon bond, a carbon-nitrogen bond or the like, to a cofactor moiety or coenzyme acting as an electron acceptor, such factors including NAD+, NADP+, cytochromes, molecular oxygen, quinones, and the like, thereby reducing the latter. In order for the reaction to continue in the direction of substrate oxidation, either fresh (i.e., unreduced) electron acceptor must be continuously supplied, or the reduced electron acceptor must be returned to its oxidized state, a process referred to herein as the "regeneration" of the electron acceptor. That, and not the particular type of oxidoreductase utilized, is the crux of the broadest claims. The genus of oxidoreductases is known, not new – thus, a *Lilly*-type inquiry as to whether enough species are disclosed to support a claim to a genus is not relevant.

While the reactants (enzyme, substrate and cofactor) may indeed vary structurally from one reaction to the next and from one system to the next, many examples of suitable reactants are well known in the art; they possess a commonality that is well recognized and understood by

those in the art: namely, participation in the catalyzed (i.e., oxidoreductase-driven) transfer of electrons from an electron donor (i.e., a substrate) to electron acceptor (e.g., a cofactor or coenzyme). The enumerated species are simply exemplary, and the additional detailed information whose absence from the specification is lamented by the Examiner (Office Action, page 5, quoted above) is simply not relevant to the written description of the claimed invention, which is directed more broadly to methods of enhancing oxidoreductase-catalyzed redox reactions in general. One of skill in the art would have such information at their fingertips, and thus it need not be included in the present specification.

The court in In re Herschler, 591 F.2d 693 (CCPA 1979), answered an analogous question in the affirmative. There, the court found that the recitation of a single steroid compound adequately supported the genus "steroidal agents," as the agents were, as a class, known. The court held that

> ...claims drawn to the use of known compounds in a manner auxiliary to the invention must have a corresponding written description only so specific as to lead one having ordinary skill in the art to that class of compounds. Occasionally, a functional recitation of those known compounds in the specification may be sufficient as that description. In Fuetterer and here, such is the case. *Id.*, 591 F.2d at 702.

Such is also the case in the present application. The present claims are drawn to the use of known compounds, oxidoreductases, in a manner auxiliary to the invention. Applicant is not claiming the compounds themselves, but rather methods of using the compounds. One of skill in the art would have recognized that one can extrapolate from the specific examples of reactions described in the specification to the broad genus presently claimed. This was explicitly predicted in the specification, and the Examiner has presented no evidence that it isn't absolutely true.

Thus, the enumerated species are sufficiently representative of the claimed genera and, when considered along with the level of skill and knowledge in the art, are sufficient to allow the skilled artisan to recognize that Applicant was in possession of the entire scope of the claims. Withdrawal of the rejection is respectfully requested.

Enablement

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. The test for "undue experimentation" is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. In fact, there are many factors to be considered when determining whether the specification is enabled and whether any necessary experimentation is "undue." They include: the breadth of the claims; the nature of the invention; the state of the prior art; the level of ordinary skill in the art; the level of predictability in the art; the amount of direction provided by the inventor; the existence of working examples; and the quantity of experimentation needed to make or use the invention.

The Examiner alleges that the specification

> does not reasonably provide enablement for (1) a method for producing an oxidized form of any organic compound or alcohol, wherein the method comprises contacting the organic compound/alcohol with a microorganism whose activity to regenerate any electron acceptor for any oxidoreductase expressed in said microorganism is enhanced by culturing said microorganism at a low dissolved oxygen concentration, or (2) a method for producing any optically active alcohol from any racemic alcohol mixture by using a microorganism capable of producing any oxidoreductase, any *C. parapsilosis* oxidoreductase, or any alcohol dehydrogenase, wherein said oxidoreductase/alcohol dehydrogenase specifically oxidizes either the (S)- or (R)-enantiomer in the racemic alcohol mixture, and wherein said microorganism is cultivated at a low dissolved oxygen concentration in order to regenerate any electron acceptor for said oxidoreductase/alcohol dehydrogenase. (Office Action, pp. 6-7)

More particularly, the Examiner alleges that the scope of the claims is not commensurate with the scope of enablement, citing the "extremely large number" of reagents encompassed by the claimed methods. (Office Action, p. 7) The Office Action goes on to list, in the carryover paragraph of pages 7-8, several categories of information that are supposedly missing from the present specification. Applicant notes that any such information needed to practice the invention is provided either in the specification or in the art. For example, information about the alcohol dehydrogenase encoded in pKK-CPA1 is provided at page 3, lines 6-24, and Examples 1-4. As

noted in Example 1, pKK-CPA1 is further described in JP-A-Hei 07-231785. Information about other oxidoreductases from other organisms is widely available in the art, as discussed above in the section regarding the written description rejection. What is known in the art need not be elaborated in the specification.

The level of skill and predictability in the relevant art are such that detailed schemes are not required for one skilled in the art to fully understand how to make and use the invention to its fullest breadth. As discussed in further detail above, oxidoreductase-catalyzed redox reactions are a known and accepted class of reactions that involve a recognized set of identified interactants with known properties. One of skill in the art would readily be able to select an oxidoreductase, select an appropriate microorganism expressing the oxidoreductase, and culture the microorganism in culture medium with a low concentration of dissolved oxygen, to enhance regeneration of the electron acceptors utilized by the oxidoreductase in the microorganism, as claimed. All of the information that one of skill in the art would need to practice the invention is either explicitly taught in the specification, or was generally known in the art.

For at least the foregoing reasons, Applicant submits that the specification, in light of the level of skill in the art and predictability as discussed above, provides ample guidance. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

The Examiner states that if the claims are amended to limit the alcohol dehydrogenase to that of pKK-CPA1, a biological deposit made in accordance with 37 CFR 1.801-1.809 may be required to comply with the enablement requirements. The claims are not presently so limited, so the issue is moot. However, Applicant points out for the record that no such deposit would be required even were they so limited. The specification states that construction of the expression vector pKK-CPA1 was performed by the methods described in JP-A-Hei 07-231785 (page 10, lines 3-7 of the present application). A computer translation of JP-A-Hei 07-231785 provided by the Japanese Patent Office on the internet is included with the information disclosure statement being submitted herewith. A nucleic acid sequence encoding the secondary alcohol dehydrogenase at issue is disclosed in the figures of JP-A-Hei 07-231785, as is the deduced amino acid sequence, and one of skill in the art would readily be able to generate the necessary

nucleic acids and microorganisms using methods known in the art. Therefore, Applicant believes that a biological deposit would not be necessary.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

Rejection Under 35 U.S.C. § 103(a)

Claims 10-20 were rejected for allegedly being obvious over Yamamoto et al. (Biosci. Biotechnol. Biochem. 63:1051-1055 (1999)) in view of Matsushita et al. (J. Bacteriol. 177:6552-6559 (1995)).

While Applicant herein provides arguments regarding the substance of Yamamoto et al., these arguments should not be considered a concession that the Yamamoto et al. reference is available as prior art against the pending claims. Note that the Yamamoto et al. reference was published in June 1999, which is after the earliest priority date of the present application, and that Hiroaki Yamamoto, the present inventor, is the first listed author of the cited reference. Applicant therefore reserves the right to remove the Yamamoto et al. document as a prior art reference in the future.

The Office Action at pages 9-10 contends that Yamamoto et al. teach the cloning and expression of *Candida parapsilosis* secondary alcohol dehydrogenase (CpSADH) in E. coli, and the use thereof to produce (R)-1,3-butanediol and 4-hydroxy-2-butanone from a racemic alcohol mixture. According to the Office Action, they further teach that "asymmetric oxidation" does not require an additional NAD+ regeneration system. The Examiner admits that the Yamamoto reference does not teach a method for producing (R)-1,3-butanediol by culturing E. coli JM109 (pKK-CPA1) at a dissolved oxygen concentration of 50%, 20%, 10% or less saturation. Matsushita et al. is cited for teaching that culturing *Acetobacter aceti* and *Gluconobacter suboxydans* bacteria under low concentrations of dissolved oxygen (low aeration conditions) results in an increase in dehydrogenase activity.

This combination fails to render the claimed invention obvious, for at least the following reasons.

The Yamamoto reference is primarily directed to a novel gene encoding a stereo-specific secondary alcohol dehydrogenase (CpSADH) and the cloning and expression thereof in E. coli, thereby providing a system for the practical synthesis of an optically pure alcohol from its racemate. At page 1055, Yamamoto et al. describe the synthesis of (R)-1,3-butanediol by E. coli expressing CpSADH. Their results purport to show that "asymmetric oxidation ... did not require additional NAD+ regeneration system." Yamamoto does not expressly identify the level of dissolved oxygen in the culture media. Finally, nothing in Yamamoto teaches or suggests that culturing microorganisms in media having a low concentration of dissolved oxygen leads to the regeneration of electron acceptors, such as NAD+, or even suggests that manipulating the oxygen levels in the media would be at all effective in increasing such regeneration.

The disclosure of Matsushita et al. fails to cure the deficiencies of Yamamoto. Matsushita et al. focused on a shift between the active and inactive forms of the enzyme, and not the cofactor/electron acceptor. Nowhere do they disclose or suggest that culturing an enzyme-expressing microorganism in a culture medium with a low concentration of dissolved oxygen, at the time the microorganism expresses the enzyme, results in the regeneration of the electron acceptor for the enzyme as required by the pending claims.

To establish a *prima facie* case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. MPEP §2142. Applicant submits that there was no motivation to combine the references cited by the Examiner, for at least the following reasons.

It is well known that commercially cultured microorganisms such as *E. coli* grow better under relatively high aeration than under low aeration conditions (see p.2, lines 16-19 of the specification as filed). Thus, there could be said to be a general bias against culturing such microorganisms under low aeration conditions. The Examiner cites Matsushita as providing a motivation to do so.

Matsushita focuses on alcohol dehydrogenases (ADH) of acetic acid bacteria of the genera *Acetobacter* and *Gluconobacter* of the family *Acetobacteraceae*. *Acetobacter* and *Gluconobacter* oxidize ethyl alcohol to acetic acid via a two-step reaction:

ethyl alcohol ----------> acetylaldehyde ----------> acetic acid
(alcohol dehydrogenase) (aldehyde dehydrogenase)

The ADH described in Matsushita is a quinohemoprotein-cytochrome c complex, composed of three subunits, that uses membrane-associated ubiquinone as an electron acceptor. Matsushita notes that the ethanol-oxidizing ability of these bacteria is easily changed or lost during cultivation; this change is associated with a genetic mutation in some strains of *Acetobacter* (pp. 6552-6553), and with changes in pH and aeration in *Gluconobacter* (p. 6553), which, Matsushita indicates, cause a shift in the ADH enzyme from an inactive to an active form. It is this observation, which is specific to the ADH of these particular microorganisms *and has nothing whatsoever to do with regeneration of electron acceptors,* that led to Matsushita's experiments using low aeration. Unless a given oxidoreductase is known or at least suspected to exist in an inactive form in the presence of high aeration, there is no motivation to try Matsushita's technique of lowering the degree of aeration in order to convert it into an active form. The Examiner has cited no basis for believing that any oxidoreductases of *Acetobacter* and *Gluconobacter,* other than ADH, also exist in an inactive form in the presence of high O_2. Nor is there any basis to assume that other microorganisms have any oxidoreductases (ADH or otherwise) with this quirk. Matsushita itself certainly doesn't provide any reason to assume it.

Indeed, the fact that Matsushita's observations are <u>not</u> generally applicable to other oxidoreductases is nicely illustrated by an experiment described in Example 3 of the present specification. The present inventors cultured *E. coli* cells expressing a recombinant oxidoreductase, growing the cells under three different levels of aeration: low (agitation at 400 rpm), medium (600 rpm) and high (800 rpm). The cells were then broken open and the enzyme's activity measured by measuring generation of NADH from the exogenous NAD+ supplied to the reaction. (Regeneration of NAD+ from NADH was presumably not occurring to any significant degree in this cell-free extract experiment, which was designed merely to test the

effect of oxygenation on the activity of the enzyme itself.) The results are shown in the middle two columns of Table 1 on page 12. If Matsushita's observations were applicable here, one would have expected to see increased activity of the enzyme in cells grown at low aeration (400 rpm) and lesser activity when the cells were grown at 600 or 800 rpm, i.e., higher aeration. *Instead, the opposite was true.* Whether expressed as units of activity per ml of culture medium (U/ml-br) or units per turbidity (U/OD, thus controlling for cell number), the enzymatic activity was higher at 600 and 800 rpm than at 400 rpm.[1] Plainly there was no activation of "inactive" enzyme occurring at low aeration, as Matsushita taught occurred with ADH in certain acetic acid-producing microorganisms. The teachings of Matsushita are thus narrowly limited to a particular enzyme produced by those particular microorganisms, and would be read that way by one of ordinary skill in the art.

Accordingly, there would be no motivation to combine the teachings of Matsushita with those of Yamamoto to arrive at the present invention, particularly in light of the bias in the art to keep oxygen levels high in order to optimize cell growth. Withdrawal of the rejection under 35 U.S.C. § 103(a) is therefore requested as to all claims.

Double Patenting Rejections

Applicant notes that claims 12-20 stand provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 40 of co-pending Application No. 10/147,003 ("the '003 application"). However, M.P.E.P. § 706.02(k) states that where two applications of different inventive entities are co-pending and the filing dates differ, a provisional rejection should be made in the later filed application if the applications have a common assignee or a common inventor. In this case, the instant application is a divisional application of 09/478,163, filed January 5, 2000. In contrast, the '003 application was filed on

[1] In contrast to these figures concerning enzyme activity measured in a cell-free extract with NAD+ supplied to the reaction, the last two columns of Table 1 illustrate the observation on which the current invention is based: intact cells, cultured in the absence of exogenously added NAD+, are better able to generate oxidized product if they are cultured in low oxygen, presumably because NAD+ is rate-limiting and is more readily regenerated from NADH in low oxygen conditions. This is despite the fact that the cells grow better at higher aeration (see second column of Table 1), and despite the fact that the enzyme in question itself is more active when the cells are grown at higher aeration (see third and fourth columns of Table 1).

May 16, 2002, thus, the '003 application is the later-filed application. Accordingly, the provisional rejection based on co-pending Application No. 10/147,003 is improper and should be withdrawn from the present application.

For the foregoing reasons, Applicant believes that the pending claims are allowable and respectfully request such action.

A check for the extension of time fee and a check for the excess claims fee are enclosed. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 14879-052002.

Respectfully submitted,

Date: July 22, 2004

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